J. Physiol. (1981), **319**, *pp.* 205–217
With 6 text-figures
Printed in Great Britain

TENSION, STIFFNESS, UNLOADED SHORTENING SPEED AND POTENTIATION OF FROG MUSCLE FIBRES AT SARCOMERE LENGTHS BELOW OPTIMUM

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(*Received 13 November 1980*)

SUMMARY

1. Unloaded shortening velocity, stiffness, and the effects of potentiators were studied to understand the basis for the shallow ascending limb (1·65–2·0 μm sarcomere length) of the sarcomere length–tension diagram of frog single fibres.

2. The velocity of externally unloaded shortening was found to be constant over most of this range. It is therefore unlikely that this part of the sarcomere length–tension diagram results from an internal force opposing shortening.

3. Stiffness was found not to vary in proportion with tension between sarcomere lengths 1·65 and 2·0 μm, nor to be constant between 2·0 and 2·2 μm, where tension is constant. By assuming a small filament compliance, the observations could be adequately modelled on the hypothesis that the variation in tension in the range of sarcomere lengths 1·65–2·0 μm was caused by variations in the number of attached cross-bridges.

4. The twitch potentiators Zn^{2+}, tetraethylammonium (TEA), nitrate and caffeine were found not to change the shape of the sarcomere length–tension diagram. Potentiation in a tetanus was less than 3% in all experiments.

5. Contractures induced by raised $[K^+]$ in the bathing solution were found to produce more tension than a tetanus beyond optimum length, insignificantly different tension near optimum length, and less tension at sarcomere lengths near 1·7 μm. An explanation is proposed for these results in terms of inhomogeneous activation and internal motion.

6. It is concluded that there is no evidence from this work that a tetanized fibre is other than maximally activated over the range of sarcomere lengths spanned by the shallow ascending limb.

INTRODUCTION

When Gordon, Huxley & Julian (1966) explained the relationship that they had found between isometric tetanic tension and sarcomere length in frog muscle fibres in terms of the sliding filament model, the region that was least obviously explained

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was the so called shallow ascending limb, the gradual increase in tension as the sarcomere length is increased from 1·65 to 2·0 μm.

If the drop in external tension below maximum is caused by the existence of an internal opposing force, then the unloaded velocity of shortening would be expected to decrease according to the force–velocity relationship. Alternatively, if the number of attached cross-bridges is less, then the tetanic stiffness should be less, accepting that the active stiffness of a carefully mounted single fibre is primarily due to attached cross-bridges, as suggested by Huxley & Simmons (1971). Somewhat contradictory and inconclusive observations of these parameters have been published, and these will be considered in the Discussion section. The first aim of this work was to clarify these measurements.

If there are indeed fewer cross-bridges attached at the shorter lengths, two possible mechanisms need to be distinguished. One is the purely mechanical interference with cross-bridge formation by the 'double overlapping', where the thin filaments from one end of a sarcomere enter the region of interaction between thick and thin filaments in the other half of the sarcomere. The second, as suggested by Rüdel & Taylor's (1971) observations of wavy myofibrils, is a failure of activation to spread throughout the core of the fibre. If this is a significant factor, the shape of the length–tension relationship would be likely to vary with fibre diameter, and with the presence of various 'potentiators', which alter either the resting membrane potential or the shape of the action potential (Sandow, 1964 Fig. 4). On this point also, contradictory results have been published (see Discussion).

These experiments, then, were performed to investigate the variation of tension, stiffness, and unloaded shortening velocity with sarcomere length, and the effect of potentiators in the range of sarcomere lengths 1·65–2·0 μm.

METHODS

The preparation and equipment was similar to that described in Julian & Morgan (1979*a*) with the following exceptions. The capacitance transducer was replaced by a semiconductor type using an AE802 element (Akers Electronics, Norway) with a preamplifier mounted on the experimental chamber. This gave a sensitivity of 1V/mN and a resonant frequency of 7 kHz, with a piece of titanium wire 5 mm long and 125 μm diameter glued to the tip of the beam for attachment of the muscle. For quick solution changes, the volume of the experimental chamber was reduced to 2 ml. by insertion of a block of Sylgard 184 solventless silicone elastomer (Dow Corning). Solutions were admitted at one end under control of solenoid valves, and removed from the other end by a vacuum pump which maintained the fluid level. As judged by dye flushing experiments, solution change was essentially complete within 1 sec. Frogs were freshly caught summer *Rana temporaria* from Ireland and were kept in a moist environment at room temperature and fed crickets.

Photomicroscopy

The muscle fibre was tetanized at four different lengths and sarcomere lengths measured photographically during contraction as previously described (Julian & Morgan 1979*a*). The microscope was adjusted so that the same section of fibre, near the centre, but outside the end-plate region, was photographed at each length. A straight line was fitted to the plot of sarcomere length against micrometer reading and this relationship was used when plotting results. Tension per unit area was found by tetanizing at 15 °C, measuring fibre width and depth at the midpoint of the width, at three points along the fibre, and estimating cross-sectional area on the assumption of elliptical cross-section. This method was described and verified by Blinks (1965).

Solutions

The Ringer solution described in Julian & Morgan (1979*a*) was modified for some of these experiments. To test the effect of Zn^{2+}, phosphate buffer was replaced with 3 mM-HEPES (Sigma) and Zn acetate (Mallinchrodt) was added to a final concentration of 50 μM. Tetraethylammonium chloride (Eastman) was added to a final concentration of 100 or 200 μM. Caffeine (Sigma) was investigated at a concentration of 1 mM. In testing the effect of nitrate, the NaCl was replaced with equimolar $NaNO_3$, while the high potassium solution contained 115 and 2·5 mM-naCl.

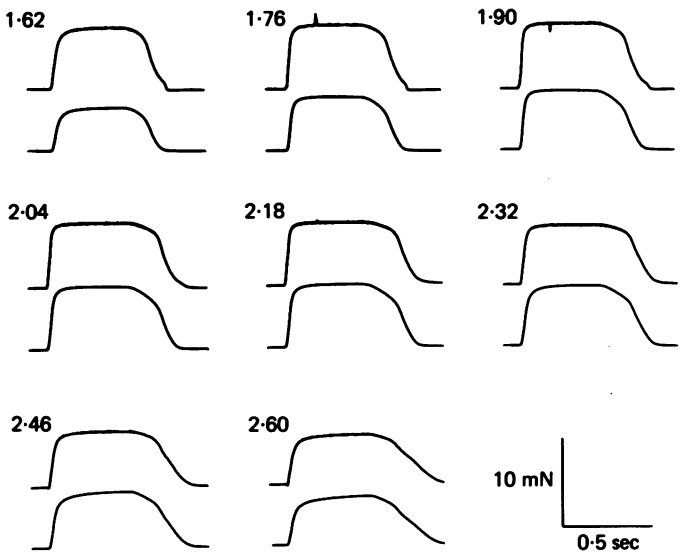

Fig. 1. Records of fixed end stiffness and tension during tetani at various sarcomere lengths. The upper trace of each pair is stiffness to an arbitrary scale while the lower is tension. The number by each pair indicates the mean active sarcomere length in μm. Expt. 80/30–1, 15 °C, maximum tetanic force 290 kN/m^2.

Protocol

Sarcomere length–tension relationships. All tetani were at regular intervals (at least 100 sec rest per second of stimulation), with control contractions every third to sixth contraction. A straight line was fitted to a plot of the control tensions against contraction number, and all tensions normalized to this line (the tension decline was never more than 5 %, and in fact tension often rose slightly). In this way it was possible to obtain length–tension plots reproducible within $\pm$1 %, except below 1·6 μm, where 2 or 3 % variations were sometimes seen.

When potentiators were used, three complete length–tension curves were obtained in each experiment, first in normal Ringer solution, then in the potentiator, and then again in normal Ringer. A minimum of 5 min, but usually 10–15 min was allowed for equilibration after solution change. The normalization procedure described above was used independently on the three sets of data and absolute potentiation defined by comparing the midpoints of the normalizing lines. The value in potentiator was compared to the mean of the two controls.

Stiffness measurement. Stiffness measurements were made using an apparatus designed and built by M. F. Julian (1979). Briefly, a small (typically 5 μm peak-to-peak amplitude) sinusoidal vibration of 1 or 2 kHz was applied to the fibre, and the resulting tension signal synchronously demodulated. The demodulated signal was then passed into a pair of one cycle gated integrators, and the outputs of those multiplexed into a sample and hold circuit so that the final output at any time was equal to the integral over the last cycle of the synchronously demodulated tension signal. This signal could then be low pass filtered if necessary, to remove noise near the vibration

frequency. A duplicate channel monitored the motor position signal to ensure that it did not vary as the muscle was tetanized. A repeatability of 1 % of the stiffness reading from tetanus to tetanus was easily achieved. Typical output is shown in the upper trace of each pair in Fig. 1.

Velocity measurement. Unloaded shortening velocity was usually measured by a 'slack test' procedure developed from that of A. V. Hill (1970, fig. 4.1) and similar to that of Edman (1979), in which step releases of various sizes, all large enough to introduce slack into the fibre, were applied, and the time required to take up the slack, i.e. begin tension recovery, was noted as shown in Fig. 3*A*, 3*B*. These lines were plotted against the step size as in Fig. 3*C*, and the slope taken as the unloaded shortening velocity. This plotting procedure involves taking a mean of the velocity over the range of sarcomere lengths involved in the extra shortening, and so the results are plotted as lines in Fig. 3*D*.

Modelling

In order to simulate the stiffness results a simple numerical model was constructed. A number (N) of cross-bridges (usually 500) were assumed evenly distributed along one half of a thick filament. At lengths beyond optimum, cross-bridges were assumed to attach throughout the overlap zone. Each cross-bridge was assumed to contribute a force of P_0/N and total force was found by adding contributions from all attached cross-bridges. Both cross-bridges and thin filaments were assumed to have a finite compliance. The total stiffness was found by simple series-parallel combination of springs, representing the cross-bridges and the sections of thin filament between cross-bridges and between the Z line and the end of the overlap zone. Thick filament length was assumed to be 1·6 μm, and the length of two thin filaments and Z line was taken as 2·05 μm.

To simulate the shallow ascending limb, the additional assumption was made that, in the region where thin filaments overlapped each other, only one third of the normal number of cross-bridges could be formed. In order that the shallow ascending limb would begin at 2·0 μm sarcomere length rather than 1·85, where the thin filament from the opposite end reaches the cross-bridge zone, this one third rule was also assumed to hold 0·075 μm ahead of the end of the thin filament (one could imagine that the filament lattice is disrupted slightly ahead of the actual 'double overlap'). This gave a close approximation to the observed length–tension curve. The stiffness was again calculated by series-parallel summation for each sarcomere length. This process was implemented on an HP-85 desktop computer enabling quick calculation for different cross-bridge and filament compliances.

The results for sarcomere lengths greater than 2·0 μm correspond with the closed form expressions derived by Ford, Huxley & Simmons (1981), but because of the additional complication of the varying force density with thin filament double overlap, a numerical method was considered simpler.

RESULTS

Length–tension relationships. Some typical records are shown in Fig. 1, (lower trace of each section) and the peak tensions are plotted and compared in Fig. 2 with the relationship found by Gordon, *et al.* (1966). The points fitted the line excellently, except in a few experiments where the same shape was apparent but shifted by 0·05–0·1 μm. In these cases, further photomicroscopy of other parts of the fibre disclosed variation in active sarcomere length along the fibre, sufficient to explain the discrepancy. With these exceptions the tension points usually fell within 2 % of the curve published by Gordon *et al.* (1966) along the plateau and shallow ascending limb. This was so for all fibres, despite cross-sectional areas ranging from 14,700 to 38,400 μm^2.

Variation of unloaded shortening velocity. Two methods were used to avoid effects of fibre aging on velocity. The first consisted of a set of releases (five to ten) within the plateau, then a set on the shallow ascending limb, followed by a second set on the plateau. In the second method, the releases from the two initial lengths were alternated. No differences were found between the two methods. Fig. 3*D* shows

results of eight experiments, three at 15 °C, two at 5 °C, and three at 1 °C. The difference in unloaded shortening velocity between 2·1–2·2 μm and 1·75–1·85 μm was 1·1 ± 1·3 % (mean, S.D.). Measurements nearer the corner at 1·67 μm gave smaller velocities, but it was difficult to be certain that this was not due to rounding of the corner caused by sarcomere length dispersion.

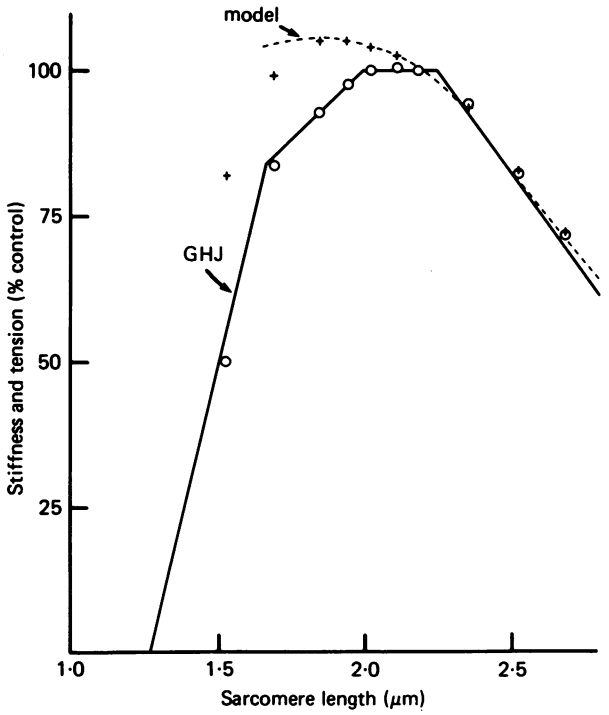

Fig. 2. Variation of tension and stiffness with sarcomere length. The circles are the fixed end tetanic tension measurements from a typical experiment using 300 msec tetani, normalized to the tension at 2·18 μm as described in the text, and plotted against mean active sarcomere length. The + signs indicate stiffness, normalized to that at 2·18 μm, measured by 2 kHz vibration. Expt 80/42–4, 5 °C, maximum tetanic tension at 15 °C = 325 kN/m^2. The (——) is the idealized sarcomere length tension diagram taken from Fig. 12 of Gordon *et al.* (1966). The (– – –) is the stiffness calculated from the model described in the text. Model parameters are: 500 cross-bridges, compliance per cross-bridge is 3·47 arbitrary units, compliance of thin filaments is 0·006 units per micron of filament length. Calculated stiffnesses were then normalised to the value at 2·18 μm to match the experimental results. These parameters are such that changing the filament compliance to zero reduced the total compliance at 2·15 μm by 30 %.

In three experiments, force-velocity curves were measured by applying ramp releases, and hyperbolae were fitted to points for tension less than 80 % P_0 (see Edman, Mulieri & Scubon-Mulieri, 1976). Again, provided the sarcomere length at which tension was measured was not less than 1·75 μm, the velocity at zero load was within 2 % of that measured on the plateau. Furthermore, no significant change in the shape of the curve, nor in the fit of the points to the hyperbola was seen in this range of lengths.

Stiffness. Records of stiffness are shown as the upper trace of each section of Fig.

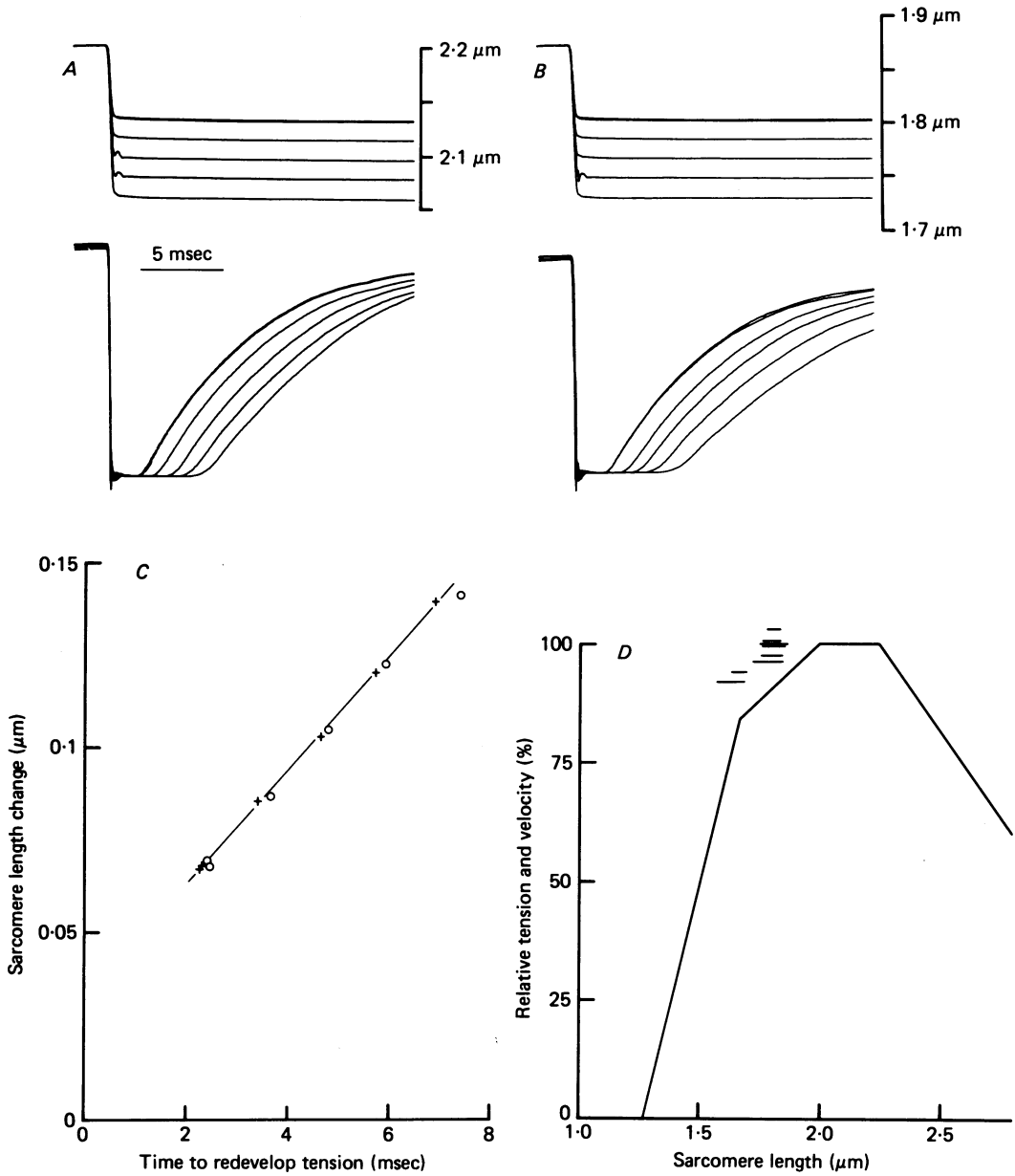

Fig. 3. Unloaded shortening velocity measurement by slack test. *A*, records for six releases from 2·2 μm mean active sarcomere length. *B*, similar releases from 1·86 μm mean active sarcomere length. Upper records show motor position calibrated in terms of mean active sarcomere length. Lower records show tension. Note that after the two largest releases in *B*, the tension rises more slowly. *C*, plot of sarcomere length change against time taken to redevelop tension, measured from parts *A* (+) and *B* (○). The line is the least squares fit to the points from part *A*. Note that the points from *B* fall very close to the line, except for the last point, 1·73 μm final sarcomere length, which departs in the 'slower' direction. Expt. 80/47, 15 °C, tetanic tension = 305 kN/m² at 2·1 μm. *D*, collected results of velocity measurement. The unloaded shortening velocity, as a percentage of plateau value is plotted against the range of sarcomere length over which is was measured, for example 1·73–1·80 μm for the measurement shown in *B*. Results from seven different fibres at temperatures ranging from 1 to 15 °C.

1 and plotted in Fig. 2. The following points should be noted as consistently seen in nine experiments. The stiffness does not fall in proportion with the force over the sarcomere length range 1·7–2·0 μm, but has approximately the same value near 1·65 μm as at 2·1 μm. Furthermore the stiffness is not constant across the plateau but rises to a broad peak between 1·8 and 1·9 μm sarcomere length. The slope across the plateau was 21 ± 5 % (mean, S.D.) per micron, in five experiments in which stiffness was measured at three or more points totally within the plateau, and the height of the peak averaged $3{\cdot}5 \pm 0{\cdot}5$ % (mean, S.D.) above the value at 2·15 μm. Beyond 2·25 μm stiffness fell approximately in proportion to tension.

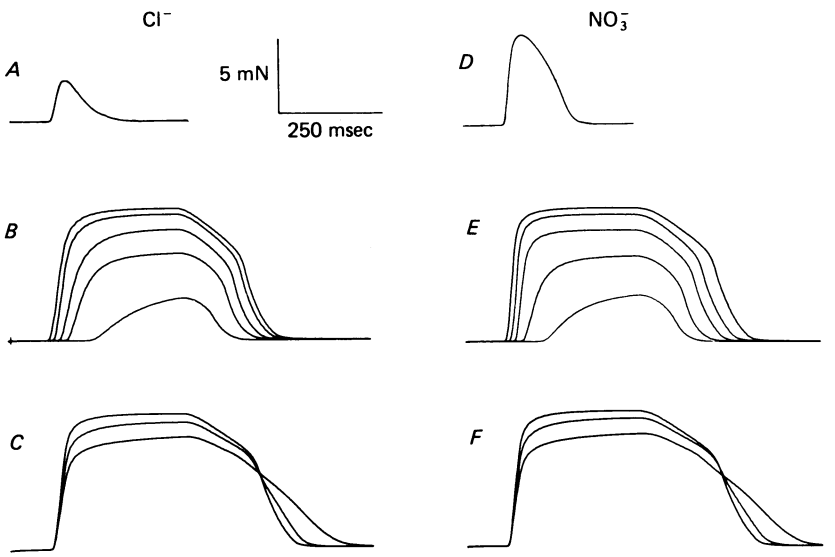

Fig. 4. Contractions in chloride (normal) *A*, *B* and *C*, and nitrate ringer, *D*, *E* and *F*. The twitch *D* is greatly enhanced and prolonged compared to *A*. In contrast, tetanic tension was practically unchanged both at fibre lengths less than optimum, *B* and *E*, and longer than optimum, *C* and *F*. Rate of rise of tension was somewhat enhanced and decay of tension retarded by NO_3^-. Sarcomere lengths (in μm) are 2·15 for twitches, 2·03, 1·88, 1·73, 1·58 and 1·43 in order of decreasing tension for *B* and *E*, and 2·18, 2·33, and 2·48 in order of decreasing tension for *C* and *F*. Expt. 80/51, 15 °C, max. tension = 280 kN/m².

Effect of potentiators. The effect of the twitch potentiators Zn^{2+} and tetraethylammonium (TEA) on the length-tension diagram was investigated in three experiments each. In addition replacement of chloride with nitrate, and addition of 1 mM-caffeine were each investigated once. The tetanic potentiation at optimum length, as defined in the Methods section was 0·7, 1·2 and 2·0 % P_0 for Zn, 1·6, 1·8 and 2·9 % for TEA, 1 % for nitrate, and a fall of 0·7 % for caffeine. It appeared that this enhancement was of slow onset (the control line for the potentiated run usually showed a slight rise) and only partially reversed after 15–30 min. (the second control was usually slightly above the first), but the small magnitude of the effect makes the significance of these observations uncertain. In contrast, twitch enhancement was always substantial, swift and reversible as shown by the records in Fig. 4 *A*, *D* (nitrate) and the + symbols of Fig. 5 (Zn).

More significantly as shown for nitrate in Fig. 4 *B*, *C*, *E*, *F*, and for Zn by the circles in Fig. 5 the shape of the length–tension curve was unchanged by the potentiators. The normalized tension at a given fibre length was highly repeatable, almost always within 1%, at least in the range of sarcomere lengths from 1·6 to 2·3 μm.

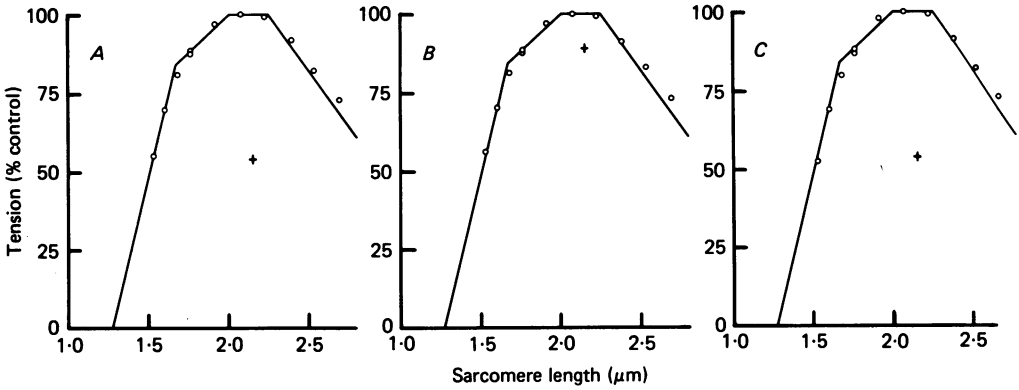

Fig. 5. The effect of Zn^{2+} on tetanic and twitch tension. An initial control run in normal Ringer solution is shown in *A*. The results in *B* were taken after equilibration in Ringer solution with 50 μM-Zn^{2+} added, and *C* is the final control run in normal Ringer. The tetanic tension measurements (○) were made in the same order for each of the three parts of this experiment. The first four tetani of each part were discarded. Every fifth contraction was a control at 2·07 μm sarcomere length. Fitting a line to the controls of each part showed that control tension rose 1·3% during the first part, fell 0·3% during the second part, and rose 2·8% during the third part of the experiment. The means of the five tetani at 2·07 μm in each part were respectively 6·70 ± 0·03, 6·83 ± 0·03 and 6·79 ± 0·04 mN. Twitch tension is shown by +. Note the pronounced twitch enhancement and the constancy of shape of the tetanic length–tension diagram. Exp. 80/35, 15 °C, maximum tension 285 kN/m^2.

It was noticed that caffeine did increase the rate of rise of tetanus, as indeed did further increases in stimulus rate above that rate required for tetanic fusion and maximum force, and that both effects become more pronounced at short lengths. A higher concentration of caffeine (3 mM), when applied to one fibre, caused a contracture and so was not used again.

In three experiments, two with Zn^{2+}, one with TEA, measurements were also made of unloaded shortening velocity by slack test at 1·75–1·85 μm sarcomere length in normal and potentiating solutions. No significant differences were found in any case, again using the normal-potentiated-normal sequence.

Potassium contractures. As a follow up to the potentiation experiments, the tension generated by depolarization in high potassium solutions was investigated. For consistent results, it was found necessary to allow long rest times between potassium contractures. Rest periods of 500 sec were used between activations. A series of several electrically stimulated tetani were followed by a K contracture, then electrical contractions until the tetanic force was repeatable, usually two or three, another K contracture and so on. In this way, and by working at 5 or 10 °C, the repeatability of tetanic tension by both forms of stimulation was within 2%. Each K contracture was normalized to the proceeding tetanus. Representative records are shown in Fig. 6.

For sarcomere lengths between 2·1 and 2·2 μm, the potassium tension during contractures exceeded tetanic tension by 0·1 $\pm$ 0·7 % (mean, S.D. of seven contractures in three fibres). At longer lengths, the K contractures gave greater tension, the actual amount depending on the duration of the electrical stimulation. When the tetanus continued until the tension peaked, the extra tension was 4·9 $\pm$ 2·1 % (mean, S.D. of two fibres, four contractures). If the tetanus was shorter, apparent extra tensions of up to 15 % could be seen. At sarcomere lengths less than 2·0 μm, the tensions were similar, though near 1·7 μm, fibres developed more tension when electrically stimulated.

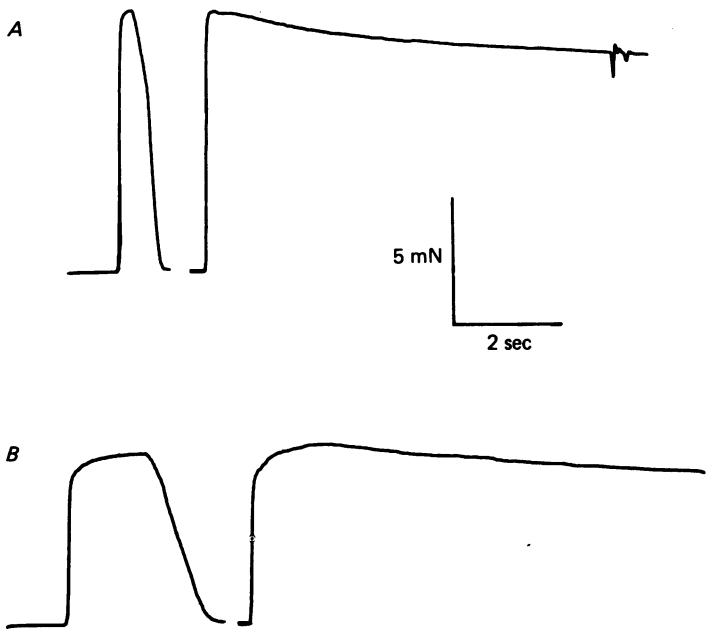

Fig. 6. Comparison of electrical tatanus (left) and high potassium contracture. *A*, mean active sarcomere length 2·15 μm. The tetanus duration was 300 msec. *B*, mean active sarcomere length 2·82 μm. Tetanus duration 1500 msec. Note the greater tension in K contractures at long lengths, but not at optimum length. If the duration of the tetanus in *B* had been the same as in *A*, the apparent extra tension would have been greater. Expt. 80/47, 10 °C, max tension = 300 kN/m^2.

DISCUSSION

The sarcomere length–tension relationship of these fibres fitted that of Gordon *et al.* (1966) closely and consistently at sarcomere lengths below 2·2 μm. The lack of variation of the sarcomere length–tension diagram with fibre area suggests that spread of activation from the surface to the centre was not a limiting factor in these fibres.

Using isotonic contractions with a load of about 6 % of maximum force, Gordon *et al.* (1966) found a decrease in shortening velocity as the sarcomere length passed from 2·0 to 1·7 μm. In one fibre, the velocity fell more rapidly than force in this range, (actually P_o–P_i the difference between isometric force at that length and the load

imposed), but in most cases it fell less than proportionately (ibid p. 184). These results were from long shortenings beginning beyond the plateau so that non-uniformity of sarcomere shortening may possibly have been a problem (see Julian & Morgan, 1979*b*). More recently, using a slack test method similar to that used here, Edman (1979) found the unloaded shortening velocity constant down to 1·65 μm. However, if the decrease in tension was entirely due to an opposing force the fractional decrease in externally unloaded shortening velocity would be much greater than the fractional decrease in force. For example, assuming an A. V. Hill (1938) hyperbola with $a = 1/4\ P_0$, $b = 1/4\ V_{max}$, then a load of 0·15 P_0 results in a velocity of 0·53 V_{max}. Hence an internal load of 15% P_0 would cause a 47% reduction in the externally unloaded shortening velocity. Such decreases have never been reported, and were never seen in these experiments. It is concluded from our unloaded shortening velocity measurements then, that only a negligible fraction of the force decrease in this region could be caused by an internal opposing force.

Using ramp releases, Bressler & Clinch (1975) found that stiffness was approximately constant above 1·82 μm and only slightly less at 1·7 μm. Our results show much less scatter than theirs but agree with the over-all conclusion that the stiffness does not fall proportionately with force in this region. However, the finding that stiffness is not constant across the plateau region calls into question the assumption, based on Huxley & Simmons (1971), that the sarcomere stiffness is entirely accounted by compliance in the cross-bridges, and not in the filaments.

To investigate the effects of varying this assumption, we constructed a simple numerical model to calculate tension and stiffness at various degrees of overlap, assuming less bridges, rather than an opposing force (see details in Methods). If the filaments were rigid, the stiffness was proportional to force, i.e. less at 1·7 than at 2·1 μm. If on the other hand, the cross-bridges were rigid, the stiffness was hyperbolic, tending to infinity as the length of unsupported thin filament tended to zero at sarcomere length 1·65 μm. It was possible to find a combination of cross-bridge and filament compliances such that the stiffness showed a broad peak at 1·8–1·9 μm, closely approximating the experimental curves as in Fig. 1. It can be seen that beyond 2·25 μm, the model stiffness approximately follows tension so that there is little disagreement with Huxley & Simmons' (1971) observation that stiffness falls in proportion with tension beyond 2·25 μm sarcomere length. The slope of the model stiffness plot across the plateau of the tension curve is 30% per μm compared to 20 ± 3% (mean, S.D.) from the experiments, and the peak stiffness was 4·0% greater than that at 2·15 μm, compared to 3·5% in the experiments.

The slope of the tension curve in the shallow ascending limb is 48%/μm, so that the stiffness is not just the summed result of fewer bridges in parallel and less filament in series, since this would cause −18% slope below 2·0 μm. This difference is caused by the non-uniform density of attached cross-bridges, with the region of decreased cross-bridge density in the double overlap zone at the relatively unstressed end of the thin filament. The differences between model and experiment could be due to a small extra compliance, in series with the fibre, which certainly cannot be ruled out in any 'end-controlled' experiment. However, such a compliance would not be expected to affect the position of the peak, which was quite sensitive to the model parameters. This would suggest that the proportions of sarcomere and filament

compliance are closely determined by these results, provided that the basic hypothesis is correct, i.e. that the slope of the stiffness curve between 2·0 and 2·25 μm is due to compliance in the filaments. The parameters used were such that reducing the compliance of the filaments to zero would have reduced the total compliance by 30 % at 2·1 μm. This does not mean that 30 % of an imposed length change would be taken up by the thin filament, as the stress along a filament is non-uniform. In fact, at 2·15 μm, about 15 % of an imposed movement would be taken up by free thin filament, i.e. between the *z*-line and the overlap zone, and the rest by the overlap network.

We are led then to the hypothesis that a small portion of the sarcomere compliance is in the thin filaments, and that this significantly affects the variation of stiffness with sarcomere length at lengths below 2·25 μm, but beyond that length, stiffness is approximately proportional for force. With this hypothesis, the measurements of stiffness are compatible with the idea that the decrease of force in the shallow ascending limb is due to a decrease in the number of bridges, rather than an opposing force.

Since these results were obtained, Ford, Huxley & Simmons (1981) have published the results of further experiments on this subject (using sarcomere lengths from 2·0 to 3·2 μm) and have concluded from step releases that the proportion of the compliance residing in the filaments is less than (and probably less than half) that concluded here. Their results for stretches however, although complicated by apparent non-linearities suggest a greater proportion than found here. Vibration might be expected to give a result intermediate between release and stretch if they were non-linear. Our main result, that our measurements of stiffness obtained by using the vibration technique cannot be taken as a true indication of the number of attached cross-bridges below 2·2 μm is unaffected by this complication.

Potentiators such as Zn^{2+}, nitrate and caffeine have traditionally been considered to increase the twitch but not the tetanus (e.g. Sandow 1964, Fig. 1, and Table 1 for other references). Their action has been described as 'prolongation but not intensification of the active state' (Sandow, ibid p. 61). More recently, however, several reports have appeared of potentiators increasing tetanic tension substantially at optimum length, e.g. Rüdel & Taylor (1971), 5–10 % extra in 2 or 3 mM-caffeine; Taylor (1976), 25 % extra in nitrate at slack length in *Xenopus* fibres whose length–tension curves were described as 'not quantitatively comparable' to frogs; and Lopez *et al.* (1977), 10 % extra in 50 mM-Zn^{2+}, and in the case of Zn^{2+} substantially changing the shape of the length–tension curve by increasing tension 60 % at 1·6 μm. The reported nitrate potentiation was also strongly length-dependent.

In contrast the present results agree with the older work reviewed by Sandow (1964), in finding only very small changes in absolute tension at optimum length, and fail to show any change in shape of the length–tension curve. Tensions below 50 % maximum were generally avoided in this study as a steady tension was not reached in a 'moderate' tetanus, i.e. 300–400 msec at 15 °C. Long tetani at very short lengths can certainly produce tension greater than indicated by the Gordon, Huxley & Julian relationship (Ramsey & Street, 1940) and caffeine, or higher stimulation rates, could reduce the duration of contraction necessary. At the sarcomere lengths of interest here though, all tetani reached a steady tension, and no potentiator significantly changed that level. The constancy of both the length–tension relationship, and

unloaded shortening velocity in agreement with Cecchi, Colomo & Lombardi (1978), support the simple idea that a tetanised fibre is fully activated, at least in this range of sarcomere lengths when a steady tension has been reached.

Hodgkin & Horowicz (1960) reported that the tension reached during a contracture induced by bathing solution containing 100 mM-K^+ was 10% greater than during a tetanic contraction. This was found both for constant [K^+] [Cl^-] product, and for normal chloride concentration as used here. The sarcomere lengths were long (2·5–2·8 μm) and Na^+ had been replaced with choline to prevent twitches. Blinks *et al.* (1978) did not confirm this (comparison of their Fig. 7 with Fig. 13 shows about 25% less tension for K^+ contractures than for electrical tetani), at 2·4 μm sarcomere length using normal Ringer solution with tetrodotoxin to block twitches.

The present results suggest that the Hodgkin and Horowicz results may well be explainable in terms of sarcomere length non-uniformity in the following way. when a fibre is activated by solution change, some part is always activated before the remainder (usually one end). Blocking of the twitch could be expected to accentuate this non-synchronous activation. On the plateau, the sarcomere length dispersion which results has little or no effect. On the descending limb, the tension is raised in a way similar to that seen in creep, but in this case substantial non-uniformity is developed quickly at the beginning of the contracture, rather than small non-uniformities gradually becoming greater as in a tetanus. The fact that shorter sarcomeres are stronger than longer ones in this range causes internal motion in such a direction as to increase the non-uniformities, and internal motion and the non-linearity of the force–velocity curve lead to increased force as explained previously (see, for example, Julian & Morgan, 1979*a*). The reduced tension in K at a sarcomere length near 1·7 μm could also be explained by dispersion and the non-linearity of the length–tension curve.

These observations, together with our findings on potentiators, in agreement with Sandow and others (Sandow, 1964, Table 1) support a simple interpretation of aequorin signals, i.e. 'that the contractile mechanism become saturated throughout the cell at a [Ca^{2+}] well below the level that is usually achieved in tetanic contractions' (Blinks, Rüdel & Taylor, 1978, p. 316), at least for sarcomere lengths beyond 1·6 μm. It should be noted that Blinks *et al.* (1978) consider this simple interpretation as being only one of several possibilities.

We conclude then that the shallow ascending limb is caused primarily by a decreased number of attached cross-bridges, probably caused by structural considerations, because we can find no convincing evidence of other than maximal activation.

This work was supported by the following grants: an N.I.H. research grant, HL-16606, from the National Heart, Lung and Blood Institute, and grants from the American Heart Association, no. 77-616, and the Muscular Dystrophy Association.

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